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EXAMINER	
MUMMERT, STEPHANIE KANE	
ART UNIT	**PAPER NUMBER**
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/684,205	CHRISTIANS ET AL.	
	Examiner	Art Unit	
	Stephanie K. Mummert, Ph.D.	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *23 February 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-7,11-26,28,30-34 and 37-45* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) *1-7,11-26,28,30-34 and 37-45* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *2/23/07*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicant's amendment filed on February 23, 2007 is acknowledged and has been entered. Claims 1-2, 11-13, 19, 31, 32 and 40 have been amended. Claims 38-39 and 44 have been canceled. Claims 1-7, 11-26, 28, 30-34, 37, 40-43 and 45 are pending.

Claims 1-7, 11-26, 28, 30-34, 37, 40-43 and 45 are discussed in this Office action.

All of the remaining amendments and arguments have been thoroughly reviewed and considered but are moot in view of the new grounds of rejection necessitated by Applicant's amendment to the claims. Any rejection not reiterated in this action has been withdrawn as being obviated by the amendment of the claims. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

This action is made NON-FINAL due to withdrawal of indication of allowable subject matter.

PREVIOUS REJECTIONS

The previous indication that claims 44 and 45 were free of the prior art and would be allowable if rewritten in independent form is withdrawn. The inclusion of the term 'consisting of' does not, in fact, render the claims allowable as free over the prior art. New grounds of rejection have been made in view of the amendment to the claims.

Information Disclosure Statement

1. The information disclosure statement (IDS) submitted on February 23, 2007 is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

Claim Interpretation

The terms "reduction oligonucleotide" and "blocking oligonucleotide" are being given the broadest reasonable interepretation in light of the specification. The terms are used interchangeably within the claims and the term "reduction oligonucleotide" is defined within the specification as "an oligonucleotide that is complementary to an unwanted nucleic acid. For example, SEQ ID NOs 1, 2 and 3 may be used as reduction oligos targeting unwanted globin mRNAs" (p. 10, lines 1-3). The term is being interpreted to broadly include any oligonucleotide complementary to an unwanted nucleic acid.

NEW GROUNDS OF REJECTION necessitated by Applicant's amendment to the claims

Claim Rejections - 35 USC § 103

1. Claims 1-2, 4, 11-12 and 15-19 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mugasimangalam et al. (US Patent 6,544,741; September 2003) in view of Kempe et al. (US Patent 4,661,450; April 1987), Baker (US Patent 5,643,780; July 1997) and Kwoh et al. (US Patent 5,055,393; October 1991) as applies to claims 1-2, 4 and 11-12 above and further in view

of Adams et al. (Nature, 1995, supplement, vol. 377, p. 3-17). Mugasimangalam discloses methods and materials for normalizing nucleic acids, using a technique called 'prime and kill' (col. 3, lines 44-51).

With regard to claim 1, Mugasimangalam discloses a method for amplifying a nucleic acid sample from blood comprising the steps:

a) hybridizing at least one reduction oligonucleotide to at least one mRNA in the sample (col. 3, lines 52-57, where a "killer primer" is designed complementary to the 3' end of highly and/or moderately expressed genes; example 1, col. 13, lines 17-22);

b) incubating the mixture with an RNAse H and subsequently inactivating the RNAse H (col. 3, lines 57-58, where RNAse H is used to digest the RNA in the DNA:RNA duplex; col. 5, lines 20-25; example 1, col. 13, lines 17-22, where the RNAse H is inactivated by heating);

d) hybridizing a primer comprising oligo dT to the RNA in the mixture and extending the primer to make single-stranded cDNA (col. 3, lines 61-62, where cDNA is synthesized from oligo dT primer; example 1, col. 13, lines 25-35); and

e) making double-stranded cDNA (col. 3, lines 61-62, where double stranded cDNA is synthesized).

With regard to claim 2, Mugasimangalam discloses an embodiment of claim 1, wherein the mRNA comprises a poly(A) tail and wherein the reduction oligonucleotide hybridizes to the unwanted RNA in the region of the mRNA that is near the 5' end of the poly(A) tail of the unwanted RNA (Figure 1A, where the reduction oligo hybridizes near the poly(A) tail; col. 3, lines 52-57, where a "killer primer" is designed complementary to the 3' end of highly and/or moderately expressed genes).

With regard to claim 4, Mugasimangalam discloses an embodiment of claim 1, wherein the RNAse H is thermolabile and inactivation is by heating (col. 5, lines 20-25).

Regarding claim 1, Mugasimangalam does not specifically teach that the nucleic acid sample is obtained from a blood sample. Kempe teaches the extraction of RNA from blood obtained from rabbit reticulocytes, establishing that blood can be a good source of RNA, and particularly of the globin sequence (col. 27, lines 39-60).

Further regarding claim 1, neither Mugasimangalam or Kempe teach the inclusion of an RNA polymerase promoter sequence in the cDNA synthesis step. Kwoh teaches the inclusion of an RNA promoter sequence in the primer used to prime cDNA synthesis. Kwoh discloses methods for determining the sex of bovine embryos (Abstract).

With regard to claim 1, Kwoh teaches d) hybridizing a primer comprising an RNA polymerase promoter sequence to the RNA in the mixture and extending the primer to make single-stranded cDNA (col. 10, lines 34-51, where a primer A including a T7 promoter is included); making double-stranded cDNA comprising a functional RNA polymerase promoter from said single stranded cDNA (col. 10, lines 34-51, where a primer A including a T7 promoter is included and the primer B is used to initiate synthesis of the second strand of cDNA; incubation with T7 polymerase results in the synthesis of RNA transcripts from the cDNA); and e) synthesizing multiple copies of labeled RNA from the double-stranded cDNA using an RNA polymerase (col. 10, lines 34-51, where RNA transcripts are generated from the cDNA).

Regarding claims 1-2, Mugasimangalam does not teach that globin mRNA is specifically targeted or the specific sequences of the reduction oligonucleotides necessary for the practice of the invention. Baker teaches that overabundance of specific highly expressed sequences in the

construction of libraries can be addressed through the inactivation and degradation of overabundant mRNA species (col. 5, lines 5-24).

With regard to claim 12, Baker discloses an embodiment of claim 10, wherein a plurality of different species of oligonucleotides are used and each species is complementary to a globin mRNA (col. 5, lines 5-24).

Baker does not teach specific subtypes of globin sequences, however Kempe discloses the isolation of globin from blood and teaches the isolation of a specific subtype of globin.

With regard to claim 11, Kempe discloses an embodiment of claim 1, wherein the unwanted nucleic acid is selected from the group consisting of alpha-1 globin, alpha-2-globin and beta globin (col. 27, lines 39-60, where beta globin is extracted from blood).

Mugasimangalam in view of Kempe discloses the limitations of claims 1-2 and 4 as recited above. With regard to claim 18, Mugasimangalam discloses an embodiment of claim 1, wherein a mixture of different sequence reduction oligonucleotides are added to the mixture (col. 15, lines 1-49, where mixtures of killer primers targeting different genes, or families of genes are disclosed, acting specifically in rat liver). However, Mugasimangalam does not teach specific sequences directed to globin sequences. Baker teaches that overabundance of specific highly expressed sequences in the construction of libraries can be addressed through the inactivation and degradation of overabundant mRNA species (col. 5, lines 5-24). Baker also does not teach specific sequences targeting globin molecules.

With regard to claim 1, Adams discloses an embodiment wherein the reduction oligo is selected from the group consisting of an oligonucleotide consisting of SEQ ID NO:1, an oligonucleotide consisting of SEQ ID NO:2 and an oligonucleotide consisting of SEQ ID NO:3

(see attached results, where sequences comprising alpha 1 globin (SEQ ID 1), alpha 2 globin (SEQ ID 2) and beta globin (SEQ ID 3) are disclosed by Adams; attached sequence results, entry 4, where AA342142 discloses the 3' end of a cDNA sequence obtained from spleen which is similar to alpha 1 globin, with sequence identity of 100% across the 23 nucleotides comprising SEQ ID NO:1; also see attached sequence results, entry 4, where AA342142 discloses the 3' end of a cDNA sequence obtained from liver which is similar to alpha 2 globin, with sequence identity of 100% across the 23 nucleotides comprising SEQ ID NO:2; also see attached sequence results, entry 4, where AA342142 discloses the 3' end of a cDNA sequence obtained from spleen which is similar to beta globin, with sequence identity of 100% across the 26 nucleotides comprising SEQ ID NO:3).

With regard to claim 15, Adams discloses an embodiment of claim 1, wherein the at least one reduction oligonucleotide consists of SEQ ID NO:1 (attached sequence results, entry 4, where AA342142 discloses the 3' end of a cDNA sequence obtained from spleen which is similar to alpha 1 globin, with sequence identity of 100% across the 23 nucleotides comprising SEQ ID NO:1; see also p. 12, Table 11, of widely expressed genes, including globin).

With regard to claim 16, Adams discloses an embodiment of claim 1, wherein the at least one reduction oligonucleotide consists of SEQ ID NO:2 (attached sequence results, entry 4, where AA342142 discloses the 3' end of a cDNA sequence obtained from liver which is similar to alpha 2 globin, with sequence identity of 100% across the 23 nucleotides comprising SEQ ID NO:2; see also p. 12, Table 11, of widely expressed genes, including globin).

With regard to claim 17, Adams discloses an embodiment of claim 1, wherein the at least one reduction oligonucleotide consists of SEQ ID NO:3 (attached sequence results, entry 4,

where AA342142 discloses the 3' end of a cDNA sequence obtained from spleen which is similar to beta globin, with sequence identity of 100% across the 26 nucleotides comprising SEQ ID NO:3; see also p. 12, Table 11, of widely expressed genes, including globin).

With regard to claim 19, Adams discloses an embodiment of claim 18, wherein the mixture comprises SEQ ID NO: 1, 2 and 3 (see attached results, where sequences comprising alpha 1 globin (SEQ ID 1), alpha 2 globin (SEQ ID 2) and beta globin (SEQ ID 3) are disclosed by Adams).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to extend the technique taught by Mugasimangalam, disclosed as capable of using virtually any prokaryotic or eukaryotic cell, to include extraction of nucleic acids from blood samples. As taught by Mugasimangalam, the technique "allows preferential elimination of highly expressed genes through cycling of the killing reaction" and "when used for subtraction, this embodiment allows enrichment of differentially expressed genes by degrading other mRNAs through killing reactions" (col. 4, lines 57-63). Mugasimangalam also teaches that "nucleic acid molecule comprises also any feasible derivative of a nucleic acid to which a nucleic acid probe can hybridize" (col. 7, lines 41-51). One of ordinary skill in the art would have recognized the universal nature of the technique taught by Mugasimangalam and also would have recognized the teaching that the method could be applicable to any cell type, and would therefore have been motivated to apply the technique to any tissue type of interest, including blood samples as taught by Kempe and Baker and additional tissues of interest, with a reasonable expectation for success.

Furthermore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to include the method of transcription amplification system

(TAS) taught by Kwoh into the method of sequence analysis taught by Mugasimangalam with a reasonable expectation for success. As taught by Kwoh, "Although target amplification is illustrated by the PCR amplification procedure, other target amplification techniques, for example the so called transcription amplification system (TAS), may equally be employed." As further noted by Kwoh, "incubation of the double stranded cDNA with T7 RNA polymerase and ribonucleotide triphosphates will result in the synthesis of RNA transcripts from the cDNA. Additional amplification can be achieved by repeating TAS on the newly synthesized RNA" (col. 10, lines 24-27 and lines 47-51). The method taught by Mugasimangalam incorporates a step of PCR amplification to determine the level or efficiency of "killing" by the stated method and by the same reasoning stated by Kwoh, it would have been obvious to include alternate amplification techniques to amplify the non-targeted sequences that remain in the sample following the "killing" or reduction step. One of ordinary skill in the art would have recognized the multiple types of amplification techniques available and would have been motivated to incorporate additional amplification techniques, in addition to the PCR amplification taught by Mugasimangalam, including the technique disclosed by Kwoh, with a reasonable expectation for success.

It also would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have targeted the abundantly expressed globin gene taught by Baker in addition to the sequences that were targeted by Mugasimangalam with a reasonable expectation for success. As taught by Mugasimangalam, "the methods and materials provided herein efficiently reduce the redundancy of highly expressed genes, and increase the relative amount of transcripts of rarely expressed genes" (col. 7, lines 5-13). As noted by Baker, "A

common problem encountered is overabundance of a particular undesired message in the library. In many cells or tissues, a particular mRNA species represents the vast majority of the total mRNA. For example, abundant mRNAs, such as those encoding globin, immunoglobulins and ovalbumin may constitute as much as 50-90% of the total poly(A)+ cytoplasmic RNA isolated from certain cell types (col. 5, lines 5-12)." Considering the abundance of expression of globin in certain cell types, specifically blood as taught by Kempe, it would have been prima facie obvious to include this target to decrease the abundance of the globin gene target in cDNA derived from a blood sample in order to enrich for less highly expressed sequences in a complex mixture of nucleic acids. Considering the teachings of both Baker and Mugasimangalam, one of ordinary skill would have recognized the benefit of greater representation of less highly expressed genes in a complex mixture of nucleic acids and would therefore have been motivated to incorporate the globin sequence taught by Baker into the preparation of the nucleic acid molecules taught by a combination of Mugasimangalam, Kempe and Kwoh with a reasonable expectation for success.

Finally, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to substitute the oligonucleotides designed to target a highly expressed gene such as globin, comprising SEQ ID NO: 1, 2 and 3 as taught by Adams for the oligonucleotide "killer primers" taught by Mugasimangalam, with a reasonable expectation for success. As taught by Baker, "A common problem encountered is overabundance of a particular undesired message in the library. In many cells or tissues, a particular mRNA species represents the vast majority of the total mRNA. For example, abundant mRNAs, such as those encoding globin, immunoglobulins and ovalbumin may constitute as much as 50-90% of the total

poly(A)+ cytoplasmic RNA isolated from certain cell types (col. 5, lines 5-12)." Considering the abundance of expression of globin in certain cell types, it would have been prima facie obvious to include this target to decrease the abundance of this target gene in the method of "killer primer" enrichment for less highly expressed sequences in the construction of a cDNA library. Given the teachings of both Baker and Mugasimangalam, one of ordinary skill would have recognized the benefit of greater representation of less highly expressed genes in the formation of cDNA libraries and would therefore have been motivated to incorporate the globin sequence taught by Adams into the reduction oligonucleotide or "killer primer" technique taught by Mugasimangalam with a reasonable expectation for success.

In the recent court decision *In Re Deuel* 34 USPQ 2d 1210 (Fed. Cir. 1995), the Court of Appeals for the Federal Circuit determined that the existence of a general method of identifying a specific DNA does not make the specific DNA obvious. Regarding structural or functional homologs, however, the Court stated,

"Normally, a *prima facie* case of obviousness is based upon structural similiarity, i.e., an established structural relationship between a prior art compound and the claimed compound. Structural relationships may provide the requisite motivation or suggestion to modify known compounds to obtain new compounds. For example, a prior art compound may suggest its homologs because homologs often have similar properties and therefore chemists of ordinary skill would ordinarily contemplate making them to try to obtain compounds with improved properties (see page 9, paragraph 4 of attached ref)."

Since the claimed primers simply represent structural homologs, which are derived from sequences suggested by the prior art as useful for primers and probes for the suppression of expression of globin genes, and concerning which a biochemist of ordinary skill would attempt to obtain alternate compounds with improved properties, the claimed primers and probes are *prima facie* obvious over the cited references in the absence of secondary considerations. Furthermore, considering the explicit teaching in the instant specification, noting, "the DNA bait

can be designed to hybridize to part or all of the RNA to be hydrolyzed. To sever the 3'-polyA tail from the rest of an mRNA, an oligonucleotide directed to a region upstream or 5' of the polyA tail may be used." The instant specification also teaches "the reduction oligo hybridized to the region that is within 50, 100 or 200 bases of the 5' end of the poly(A) tail. Hybrids of greater length may be used to generate more extensive hydrolysis. Longer DNA bait could comprise, for example: multiple oligonucleotides hybridizing to different regions of the RNA to be hydrolyzed (p. 5 of PgPub, paragraph 50). Referring specifically to the reduction of globin specific mRNA, the instant specification teaches "the reduction oligos are designed that are complementary to a region in the 3' region of one or more globin genes" (p. 9 of PgPub, paragraph 69. While the instant specification exemplifies the use of the specific reduction oligonucleotides consisting of SEQ ID NO:1 (alpha 1), SEQ ID NO:2 (alpha 2) and SEQ ID NO:3 (beta), the specification does not provide specific teaching comparing these targeted locations as providing unexpected results over sequences targeted to a different region in each of these mRNAs. Therefore, as the specification teaches that the specific location of the targeting sequence is not always restricted to only to a specific targeted location and there are no specific teachings of unexpected results associated with the specific sequences consisting of SEQ ID NO:1, 2 and 3, there is a reasonable expectation that any sequence targeting a specific sequence would be successful in depleting the targeted RNA messages, in the absence of unexpected results or other secondary considerations.

2. Claims 3 and 6 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mugasimangalam et al. (US Patent 6,544,741; September 2003) in view of Kempe et al. (US Patent 4,661,450; April 1987), Baker (US Patent 5,643,780; July 1997), Kwoh et al. (US Patent 5,055,393; October 1991) and Adams et al. (Nature, 1995, supplement, vol. 377, p. 3-17) as applies to claims 1-2, 4, 11-12 and 15-19 above and further in view of Rabin et al. (US Patent

4,745,054; May 1988). Mugasimangalam discloses methods and materials for normalizing nucleic acids, using a technique called 'prime and kill' (col. 3, lines 44-51).

Mugasimangalam in view of Kempe, Baker and Kwoh discloses the limitations of claims 1-2, 4, 11-12 and 15-19 as recited in the 103 rejection stated above. Mugasimangalam teaches the inactivation of RNAse H with heat, after the digestion of the RNA portion of the RNA:DNA hybrid; however, Mugasimangalam does not disclose the limitations of claims 3 or 6, where the ribonuclease is inactivated through organic extraction.

Rabin discloses a method of detecting an analyte in a sample after an enzymatic reaction (Abstract).

With regard to claim 3, Rabin discloses an embodiment of claim 1, wherein the RNAse H is inactivated by depleting RNAse H from the mixture (experimental section, II. 'stopping the reaction' heading, col. 11, lines 31-46).

With regard to claim 6, Rabin discloses an embodiment of claim 1, wherein the RNAse H is inactivated by separating the RNAse H from the nucleic acid by organic extraction (experimental section, II. 'stopping the reaction' heading, col. 11, lines 31-46).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to extend the inactivation of RNAse H taught by Mugasimangalam by using alternate techniques known in the art at the time the invention was made. These techniques are varied and are designed to remove or inactivate an enzyme that may interfere with downstream steps after reaction using the enzyme (e.g., ribonuclease). Rabin teaches of depletion of a ribonuclease after conducting a step directed to Enzyme catalysed production of Cp Riboflavin using phenol extraction. While the method taught by Rabin uses a ribonuclease as part of a large

enzymatic reaction, the concept taught by Rabin of the necessity of stopping the reaction is shared between Rabin and Mugasimangalam. As taught by Rabin, "It was essential at this point to inactivate the ribonuclease in the incubation mixture as exposure of the product to the enzyme in an aqueous environment would result in its breakdown to cytidine 3'-phosphate and riboflavin" (col. 11, lines 31-46). While the ribonuclease taught by Mugasimangalam is used to digest RNA, it is important to inactivate the enzyme before further steps are conducted to avoid digestion of non-targeted sequences that remain in the sample following reaction with the blocking oligonculeotide. One of ordinary skill in the art at the time the invention was made would have been motivated to consider alternate techniques to deplete, remove or inactivate the RNAse H enzyme prior to further processing of the mRNA sample with a reasonable expectation of success.

3. Claims 3, 5 and 7 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mugasimangalam et al. (US Patent 6,544,741; September 2003) in view of Kempe et al. (US Patent 4,661,450; April 1987), Baker (US Patent 5,643,780; July 1997), Kwoh et al. (US Patent 5,055,393; October 1991) and Adams et al. (Nature, 1995, supplement, vol. 377, p. 3-17) as applies to claims 1-2, 4, 11-12 and 15-19 above and further in view of Stamatoyannapoulos et al. (2003/0170689; September 2003). Mugasimangalam discloses methods and materials for normalizing nucleic acids, using a technique called 'prime and kill' (col. 3, lines 44-51).

With regard to claim 3, Stamatoyannapoulos discloses an embodiment of claim 1, wherein the RNAse H is inactivated by depleting RNAse H from the mixture (p. 18, paragraph

0188, where reaction was 'cleaned up' using a column to remove enzymes, etc. from a reaction used to blunt a DNA molecule).

With regard to claim 5, Stamatoyannapoulos discloses an embodiment of claim 1, wherein the RNAse H is inactivated by addition of EDTA to the mixture (p. 17, paragraph 0155, example 7, where EDTA is added to quench a reaction).

With regard to claim 7, Stamatoyannapoulos discloses an embodiment of claim 1, wherein the RNAse H is removed by separating the RNA from the RNAse H by column purification (p. 18, paragraph 0188, where reaction was 'cleaned up' using a column to remove enzymes, etc. from a reaction used to blunt a DNA molecule).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to extend the inactivation of RNAse H taught by Mugasimangalam by using alternate techniques known in the art at the time the invention was made. These techniques are varied and are designed to remove or inactivate an enzyme that may interfere with downstream steps after reaction using the enzyme (e.g., Klenow or polymerase). Stamatoyannapoulos teaches multiple instances of inactivation or depletion of enzyme(s) following particular steps in a multi-step protocol. The method taught by Stamatoyannapoulos, includes multiple steps, including labeling of DNA fragments using a Klenow fragment, followed by stopping the reaction by the addition of EDTA (p. 17, paragraph 0155) and a 'clean up' step after blunting and tailing probes for hybridization to a microarray by processing on a column (p. 18, paragraph 0188). While neither of these steps directly involves the inactivation or depletion of a ribonuclease, such as RNAse H, the steps demonstrate the state of the art with regards to different techniques directed to inactivation or depletion of enzymes following an enzymatic

reaction. The ribonuclease of Mugasimangalam is used to digest RNA, it is important to inactivate the enzyme before further steps are conducted to avoid digestion of non-targeted sequences that remain in the sample following reaction with the blocking oligonculeotide. One of ordinary skill in the art at the time the invention was made would have been motivated to consider alternate techniques, such as those taught by Stamatoyannapoulos to deplete, remove or inactivate the an enzyme such as RNAse H prior to further processing of the mRNA sample with a reasonable expectation of success.

4. Claims 20-23 are rejected under 35 U.S.C. 103(a) as being obvious over Mugasimangalam et al. (US Patent 6,544,741; September 2003) in view of Kempe et al. (US Patent 4,661,450; April 1987), Baker (US Patent 5,643,780; July 1997), Kwoh et al. (US Patent 5,055,393; October 1991) and Adams et al. (Nature, 1995, supplement, vol. 377, p. 3-17) as applies to claims 1-2, 4, 11-12 and 15-19 above and further in view of Augello et al. (US Patent 6,602,718; August 2003). Mugasimangalam discloses methods and materials for normalizing nucleic acids, using a technique called 'prime and kill' (col. 3, lines 44-51).

Mugasimangalam teaches the limitations of claims 1-2, 4, 11-12 and 15-19 as recited in the 103 rejection stated above. However, Mugasimangalam does not specifically teach that the nucleic acid sample is isolated from blood or that the blood was collected in a container containing an RNA stabilizing agent. Augello teaches a container specifically designed for the collection of blood and includes a variety of RNA stabilizing agents (Abstract; col. 3, lines 44-58).

With regard to claim 20, Augello discloses an embodiment of claim 1, wherein said nucleic acid sample from blood is obtained from blood that was collected in a container containing an RNA stabilizing agent (Abstract; col. 3, lines 44-58).

With regard to claim 21, Augello discloses an embodiment of claim 20, wherein said RNA stabilizing agent is selected from the group consisting of cationic compounds, detergents, chaotropic salts, ribonuclease inhibitors, chelating agents, and mixtures thereof (col. 3, lines 44-58, where the nucleic acid stabilizing agent can be a detergent, a chaotropic salt, RNAse inhibitors, chelating agents, or mixtures; see also col. 6, line 43 to col. 8, line 59).

With regard to claim 22, Augello discloses an embodiment of claim 20, wherein said RNA stabilizing agent is selected from the group consisting of phenol, chloroform, acetone, alcohols and mixtures thereof (col. 6, line 51 to col. 7 line 18).

With regard to claim 23, Augello discloses an embodiment of claim 20, wherein said nucleic acid sample from blood is obtained from blood that was collected in a container containing an RNA stabilizing agent and wherein said RNA stabilizing agent is selected from the group consisting of mercapto-alcohols, dithiothreitol (DTT) and mixtures thereof (col. 6, line 51 to col. 7 line 18).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to incorporate the teachings of Augello into the method taught by Mugasimangalam with a reasonable expectation for success. As taught by Augello, upon evaluating the stabilization of nucleic acids obtained using the collection container with RNA stabilizers, "The real-time PCR results show that in the unpreserved EDTA blood, the transcript level decreases over time (indicated by the increasing Ct value in the TaqMan analysis) up to a

degree of degradation after 7 to 10 days at which point the mRNA is no longer detectable. On the other hand the GAPDH mRNA in the preserved samples does not show any decrease in copy number" (col. 11, lines 26-33). Therefore, the inclusion of the RNA stabilizer and collection container taught by Augello provides the benefit of increased stability over time, allowing for analysis of RNA samples over a longer time frame. One of ordinary skill in the art would have recognized the benefit of blood collection in the presence of RNA stabilizers, who would have therefore been motivated to incorporate the teachings of Augello to the method taught by Mugasimangalam with a reasonable expectation for success.

5. Claims 31-34 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mugasimangalam et al. (US Patent 6,544,741; September 2003) in view of Kempe et al. (US Patent 4,661,450; April 1987), Baker (US Patent 5,643,780; July 1997) and Kwoh et al. (US Patent 5,055,393; October 1991) and Adams et al. (Nature, 1995, supplement, vol. 377, p. 3-17) as applies to claims 1-2, 4, 11-12 and 15-19 above and further in view of Baker et al. (US Patent 5,643,780; July 1997), Rampersad et al. (US Patent 5,830,712; November 1998) and Winzeler et al. (Methods in Enzymology, vol. 306, 1999, p. 3-18). Mugasimangalam discloses methods and materials for normalizing nucleic acids, using a technique called 'prime and kill' (col. 3, lines 44-51).

Mugasimingalam in view of Kempe, Baker and Kwoh discloses the details of claims 1-2, 4, 11-12 and 15-19 as recited in the 103 rejection stated above. However, Mugasimingalam does not teach the targeting of globin sequences specifically. Baker teaches that overabundance of

specific highly expressed sequences in the construction of libraries can be addressed through the inactivation and degradation of overabundant mRNA species (col. 5, lines 5-24).

With regard to claim 11, Kempe discloses an embodiment of claim 1, wherein the unwanted nucleic acid is selected from the group consisting of alpha-1 globin, alpha-2-globin and beta globin (col. 27, lines 39-60, where beta globin is extracted from blood).

Regarding claim 31, none of Mugasimingalam, Kempe, Baker and Kwoh teaches specific sequences of globin which are targeted using the reduction oligonucleotides.

With regard to claim 31, Adams discloses an embodiment wherein the reduction oligo is selected from the group consisting of an oligonucleotide consisting of SEQ ID NO:1, an oligonucleotide consisting of SEQ ID NO:2 and an oligonucleotide consisting of SEQ ID NO:3 (see attached results, where sequences comprising alpha 1 globin (SEQ ID 1), alpha 2 globin (SEQ ID 2) and beta globin (SEQ ID 3) are disclosed by Adams and any one of these could be selected and be expected to function; see also attached sequence results, entry 4, where AA342142 discloses the 3' end of a cDNA sequence obtained from spleen which is similar to alpha 1 globin, with sequence identity of 100% across the 23 nucleotides comprising SEQ ID NO:1; also see attached sequence results, entry 4, where AA342142 discloses the 3' end of a cDNA sequence obtained from liver which is similar to alpha 2 globin, with sequence identity of 100% across the 23 nucleotides comprising SEQ ID NO:2; also see attached sequence results, entry 4, where AA342142 discloses the 3' end of a cDNA sequence obtained from spleen which is similar to beta globin, with sequence identity of 100% across the 26 nucleotides comprising SEQ ID NO:3).

Mugasimingalam does not teach the extension of the reduction oligonucleotide by a polymerase, or the removal of the reduction oligonucleotide after reaction with the target nucleic acid as recited in claims 13-14 and 31-34.

Rampersad teaches a method for inactivating undesirable members in a sample which includes desirable and undesirable sequences in a mixture (Abstract), including these steps, as follows.

Further, with regard to claim 31, Rampersad discloses a method for amplifying a nucleic acid sample comprising:

a) providing a nucleic acid sample (col. 4, lines 1-17; col. 5, lines 9-33);

b) hybridizing at least one reduction oligonucleotide to at least one mRNA in the sample generating reduction oligonucleotide:mRNA complexes (col. 4, lines 1-17, where the DNA:RNA hybrids form between the mRNA and the oligo blocker; example 1, col. 5, lines 9-33, where an oligonucleotide blocker specific to rat NPY Y1 sequence was incubated with total rat hypothalamus RNA);

c) removing said complexes from the sample (col. 6, lines 10-23).

With regard to claim 32, Rampersad discloses an embodiment of claim 31, wherein said complexes are removed from the sample by affinity purification (col. 6, lines 10-23).

With regard to claim 33, Rampersad discloses an embodiment of claim 31, wherein said reduction oligonucleotide comprises biotin and said complexes are removed from the sample by hybridization to a solid support (col. 6, lines 10-23, where the oligonucleotide blocker is removed using biotin/streptavidin bead technology).

With regard to claim 34, Rampersad discloses an embodiment of claim 33, wherein said solid support comprises streptavidin (col. 6, lines 10-23, where the oligonucleotide blocker is removed using biotin/streptavidin bead technology).

Regarding claim 31, neither Mugasimangalam, Kempe, Baker, Kwoh or Rampersad teaches the amplification and labeling of cDNA using random primers and hybridizing the labeled primers to an array of nucleic acid probes. Winzeler teaches the hybridization of nucleic acids to a solid support to generate a hybridization pattern to determine expression levels (Abstract).

Regarding claim 31, Winzeler teaches a method comprising:

d) amplifying at least one target RNA remaining in the sample by a method comprising adding random primers to the sample, extending the random primers to make cDNA and labeling the cDNA (p. 14, 'microarray target preparation by single-round reverse transcription' heading, where it is noted that random hexamers or nonamers can be used in place of oligodT to prime cDNA synthesis); and

e) hybridizing the labeled cDNA to an array of nucleic acid probes and analyzing a resulting hybridization pattern (p. 14, 'hybridization and washing' heading, where labeled target is hybridized to a microarray for times ranging from 2 to 20 hours).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to incorporate the method of inactivation and blocking taught by Rampersad to complement the "killer primer" method taught by Mugasimangalam with a reasonable expectation for success. As taught by Rampersad, the term "undesired nucleic acids is used herein to denote nucleic acids in a sample that interfere with the use of the sample. Undesirable

nucleic acids may include known or abundant members of a nucleic acid family that block the study, isolation or processing of desired novel or less abundant members of the same family" (col. 2, lines 30-34). While, Mugasimangalam teaches that "the methods and materials provided herein efficiently reduce the redundancy of highly expressed genes, and increase the relative amount of transcripts of rarely expressed genes" (col. 7, lines 5-13). As both Rampersad and Mugasimangalam teach related methods for the reduction of abundant target sequences in complex mixtures of oligonucleotides, it would have been obvious to one of ordinary skill in the art at the time the invention was made to incorporate the teachings of Rampersad, including the removal of the blocking oligonucleotide following reaction with the nucleic acid sample and potentially designing the killer primer to be extendable by a polymerase, instead of priming synthesis using oligo d(T), as taught by Mugasimangalam. One of ordinary skill would have recognized the benefit of modifying a technique to fit a given set of experimental conditions and would have therefore been motivated to consult similar methods in the art in order to arrive at the most optimal experimental conditions to achieve the stated goal.

Furthermore, it also would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to analyze the amplified sample, enriched for less abundant transcripts, through hybridization to an oligonucleotide array. According Winzeler, "miniature nucleic acid arrays, often called 'DNA chips' or 'microarrays', offer opportunities to collect much of the same data that can be obtained with standard molecular biology hybridization methods but in a highly parallel fashion. These microarrays contain dense collections of nucleic acids [either polymerase chain reaction (PCR) products or oligonucleotides] that are either synthesized or deposited at fixed locations on specially prepared glass slides" (p. 3, first

paragraph). The increased ability for parallel analysis allowed by microarray hybridization provides the benefit of savings in time and in reagents as compared to analysis of cDNA or genomic DNA libraries using conventional molecular biology techniques.

However, it is noted, regarding the construction of DNA microarrays, Winzeler teaches that "nucleic acid probes (usually 500 to 2000 bases in size) are generated by PCR-amplifying plasmid library inserts (using primers complementary to the vector portion of the library) or portions of genomic DNA (using primers designed specifically for the open reading frames or genes of interest). The PCR products are then deposited, usually using a robotic microspotting device, at defined locations on a glass slide" (p. 5, top paragraph). Keeping in mind that spotting of plasmid library inserts is one of the methods of microarray construction and considering the teachings of Baker, that "A common problem encountered is overabundance of a particular undesired message in the library" (col. 5, lines 5-12), the library source for a microarray could represent an important aspect of experimental design. An overabundance of a specific message within a library, particularly if the library is used for spotting of a microarray prior to analysis using the same array could result in a loss of less highly expressed messages within the library present on the array due to an over-representation of the more highly expressed gene(s) in the library. Therefore, it would have been prima facie obvious to include targeting of the specific globin sequence targets taught by Kempe above in addition to targeting additional sequences disclosed by Mugasimangalam in order to enrich for less highly expressed sequences in a complex mixture of nucleic acids prior to microarray analysis using the method disclosed by Winzeler to arrive at the claimed invention with a reasonable expectation for success to provide a more reliable sample of the nucleic acids present in the blood sample.

6. Claims 24-26, 28 and 30-31 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mugasimangalam et al. (US Patent 6,544,741; September 2003) in view of Kempe et al. (US Patent 4,661,450; April 1987), Baker (US Patent 5,643,780; July 1997), Winzeler et al. (Methods in Enzymology, vol. 306, 1999, p. 3-18) and Adams et al. (Nature, 1995, supplement, vol. 377, p. 3-17). Mugasimangalam discloses methods and materials for normalizing nucleic acids, using a technique called 'prime and kill' (col. 3, lines 44-51).

With regard to claim 24, Mugasimangalam discloses a method for analyzing a nucleic acid sample comprising:

a) providing a first nucleic acid sample (col. 3, lines 52-57; col. 11, lines 5-10, where the biological sample is from an animal, plant, bacterium or virus or can be from any eukaryotic or prokaryotic cell);

b) blocking amplification of sequences in the nucleic acid sample by hybridizing a reduction oligonucleotide to said mRNA sequences to form an RNA:DNA hybrid and digesting the RNA:DNA hybrids (col. 3, lines 52-57, where a "killer primer" is designed complementary to the 3' end of highly and/or moderately expressed genes; example 1, col. 13, lines 17-22; col. 3, lines 57-58, where RNAse H is used to digest the RNA in the DNA:RNA duplex; col. 5, lines 20-25; example 1, col. 13, lines 17-22);

c) amplifying unblocked nucleic acid sequences to produce an amplified nucleic acid sample (col. 4, lines 21-24, where an embodiment includes testing the efficiency of killing by PCR amplification; col. 5, lines 20-25);

With regard to claim 30, Mugasimangalam discloses an embodiment of claim 24, wherein said target mRNAs are greater than 20% of the first nucleic acid sample and wherein said target mRNAs are less than 20% of the amplified nucleic acid sample (col. 6, lines 5-13, where the target genes comprise 0.5 to 90% pf all of the genes in the sample; col. 14, lines 5-7, where targeted high copy number genes were not detected after treatment with the "killer primer" method).

With regard to claim 31, Mugasimangalam discloses a method for amplifying a nucleic acid sample comprising:

a) providing a nucleic acid sample (col. 3, lines 52-57; col. 11, lines 5-10, where the biological sample is from an animal, plant, bacterium or virus or can be from any eukaryotic or prokaryotic cell);

b) hybridizing at least one reduction oligonucleotide to at least one mRNA in the sample generating reduction oligonucleotide:mRNA complexes (col. 3, lines 52-57, where a "killer primer" is designed complementary to the 3' end of highly and/or moderately expressed genes; example 1, col. 13, lines 17-22);

c) removing said complexes from the sample (col. 3, lines 57-58, where RNAse H is used to digest the RNA in the DNA:RNA duplex; col. 5, lines 20-25; example 1, col. 13, lines 17-22); and

d) amplifying at least one target RNA remaining in the sample (col. 4, lines 21-24, where an embodiment includes testing the efficiency of killing by PCR amplification; col. 5, lines 20-25).

With regard to claim 35, Mugasimangalam discloses an embodiment of claim 31, wherein the RNA is amplified by mixing with random primers, extending the random primers to

make cDNA (col. 4, lines 39-42, where the cDNA is primed using random primers or oligo d(T) for first strand synthesis)..

Regarding claim 24 and 31, Mugasimangalam does not specifically teach obtaining the nucleic acid sample from blood, targeting of globin mRNA specifically in the complex mixture, does not teach the specific sequences of the targeting probes/primers and also does not teach contacting the amplified nucleic acid sample with a solid support to generate a hybridization pattern. Mugasimangalam also does not teach the labeling of the cDNA.

With regard to claim 24 and 31, Adams discloses an embodiment wherein the reduction oligo is selected from the group consisting of an oligonucleotide consisting of SEQ ID NO:1, an oligonucleotide consisting of SEQ ID NO:2 and an oligonucleotide consisting of SEQ ID NO:3 (see attached results, where sequences comprising alpha 1 globin (SEQ ID 1), alpha 2 globin (SEQ ID 2) and beta globin (SEQ ID 3) are disclosed by Adams and any one of these could be selected and be expected to function; see also attached sequence results, entry 4, where AA342142 discloses the 3' end of a cDNA sequence obtained from spleen which is similar to alpha 1 globin, with sequence identity of 100% across the 23 nucleotides comprising SEQ ID NO:1; also see attached sequence results, entry 4, where AA342142 discloses the 3' end of a cDNA sequence obtained from liver which is similar to alpha 2 globin, with sequence identity of 100% across the 23 nucleotides comprising SEQ ID NO:2; also see attached sequence results, entry 4, where AA342142 discloses the 3' end of a cDNA sequence obtained from spleen which is similar to beta globin, with sequence identity of 100% across the 26 nucleotides comprising SEQ ID NO:3).

Kempe teaches the extraction of RNA from blood obtained from rabbit reticulocytes, establishing that blood can be a good source of RNA, and particularly of the globin sequence (col. 27, lines 39-60).

Baker teaches that overabundance of specific highly expressed sequences in the construction of libraries can be addressed through the inactivation and degradation of overabundant mRNA species (col. 5, lines 5-24). Baker also teaches that a specific example of an overexpressed gene includes globin (col. 5, lines 5-24).

Neither Mugasimangalam or Baker teach the hybridization of nucleic acids to a solid support or amplifying the target RNA by a method comprising adding random primers to the sample. Winzeler teaches the hybridization of nucleic acids to a solid support to generate a hybridization pattern to determine expression levels (Abstract).

Regarding claim 31, Winzeler teaches a method comprising:

d) amplifying at least one target RNA remaining in the sample by a method comprising adding random primers to the sample, extending the random primers to make cDNA and labeling the cDNA (p. 14, 'microarray target preparation by single-round reverse transcription' heading, where it is noted that random hexamers or nonamers can be used in place of oligodT to prime cDNA synthesis); and

e) hybridizing the labeled cDNA to an array of nucleic acid probes and analyzing a resulting hybridization pattern (p. 14, 'hybridization and washing' heading, where labeled target is hybridized to a microarray for times ranging from 2 to 20 hours).

With regard to claim 25, Winzeler discloses an embodiment of claim 24, further comprising detecting the presence or absence of hybridization of said amplified nucleic acid

sample to said nucleic acid probes on said solid support (p. 16, 'data analysis' heading, where the presence or absence of hybridization is determined for each array element).

With regard to claim 26, Winzeler discloses an embodiment of claim 24, further comprising labeling said amplified nucleic acid sample (p. 11-14, where a variety of labeling techniques and circumstances are described in detail).

With regard to claim 28, Winzeler discloses an embodiment of claim 24, wherein said unblocked nucleic acid sequences are non-specifically amplified by in vitro transcription (p. 13-14, 'generating labeled target by in vitro transcription' heading).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have targeted the abundantly expressed globin gene taught by Baker in addition to the sequences that were targeted by Mugasimangalam with a reasonable expectation for success. As taught by Mugasimangalam, "the methods and materials provided herein efficiently reduce the redundancy of highly expressed genes, and increase the relative amount of transcripts of rarely expressed genes" (col. 7, lines 5-13). As noted by Baker, "In many cells or tissues, a particular mRNA species represents the vast majority of the total mRNA. For example, abundant mRNAs, such as those encoding globin, immunoglobulins and ovalbumin may constitute as much as 50-90% of the total poly(A)+ cytoplasmic RNA isolated from certain cell types (col. 5, lines 5-12)." Considering the abundance of expression of globin in certain cell types, specifically blood as taught by Kempe and Baker, it would have been prima facie obvious to include this target to decrease the abundance of the globin gene target in cDNA derived from a blood sample in order to enrich for less highly expressed sequences. Considering the teachings of both Baker and Mugasimangalam, one of ordinary skill would have recognized the benefit of

greater representation of less highly expressed genes in a complex mixture of nucleic acids isolated from blood and would therefore have been motivated to incorporate the globin sequence taught by Baker into the preparation of the nucleic acid molecules taught by a combination of Mugasimangalam and Kempe with a reasonable expectation for success.

Furthermore, it also would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to analyze the amplified sample, enriched for less abundant transcripts, through hybridization to an oligonucleotide array. According Winzeler, "miniature nucleic acid arrays, often called 'DNA chips' or 'microarrays', offer opportunities to collect much of the same data that can be obtained with standard molecular biology hybridization methods but in a highly parallel fashion. These microarrays contain dense collections of nucleic acids [either polymerase chain reaction (PCR) products or oligonucleotides] that are either synthesized or deposited at fixed locations on specially prepared glass slides" (p. 3, first paragraph). The increased ability for parallel analysis allowed by microarray hybridization provides the benefit of savings of time and reagents as compared to analysis of cDNA or genomic DNA libraries using conventional molecular biology techniques.

However, it is also noted regarding the construction of DNA microarrays, Winzeler teaches that "nucleic acid probes (usually 500 to 2000 bases in size) are generated by PCR-amplifying plasmid library inserts (using primers complementary to the vector portion of the library) or portions of genomic DNA (using primers designed specifically for the open reading frames or genes of interest). The PCR products are then deposited, usually using a robotic microspotting device, at defined locations on a glass slide" (p. 5, top paragraph). Keeping in mind that spotting of plasmid library inserts is one of the methods of microarray construction and

considering the teachings of Baker, that "A common problem encountered is overabundance of a particular undesired message in the library" (col. 5, lines 5-12), the library source for a microarray could represent an important aspect of experimental design. An overabundance of a specific message within a library, particularly if the library is used for spotting of a microarray prior to analysis using the same array could result in a loss of less highly expressed messages within the library present on the array due to an over-representation of the more highly expressed gene(s) in the library. Therefore, it would have been prima facie obvious to include targeting of the specific globin sequence targets taught by Kempe above in addition to targeting additional sequences disclosed by Mugasimangalam in order to enrich for less highly expressed sequences in a complex mixture of nucleic acids prior to microarray analysis using the method disclosed by Winzeler to arrive at the claimed invention with a reasonable expectation for success to provide a more reliable sample of the nucleic acids present in the blood sample.

In the recent court decision *In Re Deuel* 34 USPQ 2d 1210 (Fed. Cir. 1995), the Court of Appeals for the Federal Circuit determined that the existence of a general method of identifying a specific DNA does not make the specific DNA obvious. Regarding structural or functional homologs, however, the Court stated,

"Normally, a *prima facie* case of obviousness is based upon structural similiarity, i.e., an established structural relationship between a prior art compound and the claimed compound. Structural relationships may provide the requisite motivation or suggestion to modify known compounds to obtain new compounds. For example, a prior art compound may suggest its homologs because homologs often have similar properties and therefore chemists of ordinary skill would ordinarily contemplate making them to try to obtain compounds with improved properties (see page 9, paragraph 4 of attached ref)."

Since the claimed primers simply represent structural homologs, which are derived from sequences suggested by the prior art as useful for primers and probes for the suppression of expression of globin genes, and concerning which a biochemist of ordinary skill would attempt

to obtain alternate compounds with improved properties, the claimed primers and probes are *prima facie* obvious over the cited references in the absence of secondary considerations. Furthermore, considering the explicit teaching in the instant specification, noting, "the DNA bait can be designed to hybridize to part or all of the RNA to be hydrolyzed. To sever the 3'-polyA tail from the rest of an mRNA, an oligonucleotide directed to a region upstream or 5' of the polyA tail may be used." The instant specification also teaches "the reduction oligo hybridized to the region that is within 50, 100 or 200 bases of the 5' end of the poly(A) tail. Hybrids of greater length may be used to generate more extensive hydrolysis. Longer DNA bait could comprise, for example: multiple oligonucleotides hybridizing to different regions of the RNA to be hydrolyzed (p. 5 of PgPub, paragraph 50). Referring specifically to the reduction of globin specific mRNA, the instant specification teaches "the reduction oligos are designed that are complementary to a region in the 3' region of one or more globin genes" (p. 9 of PgPub, paragraph 69. While the instant specification exemplifies the use of the specific reduction oligonucleotides consisting of SEQ ID NO:1 (alpha 1), SEQ ID NO:2 (alpha 2) and SEQ ID NO:3 (beta), the specification does not provide specific teaching comparing these targeted locations as providing unexpected results over sequences targeted to a different region in each of these mRNAs. Therefore, as the specification teaches that the specific location of the targeting sequence is not always restricted to only to a specific targeted location and there are no specific teachings of unexpected results associated with the specific sequences consisting of SEQ ID NO:1, 2 and 3, there is a reasonable expectation that any sequence targeting a specific sequence would be successful in depleting the targeted RNA messages, in the absence of unexpected results or other secondary considerations.

7. Claims 37-39 and 42 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lockhart et al. (Nat Biotech, 1996, vol. 14, p. 1675-1680) in view of Rampersad et al. (US Patent

5,830,712; November 1998), Kempe (US Patent 4,661,450; April 1987), Baker et al (US Patent 5,643,780; July 1997) and Adams et al. (Nature, 1995, supplement, vol. 377, p. 3-17). Lockhart teaches hybridization to small, high-density oligonucleotide arrays, designed using a combination of photolithography and oligonucleotide synthesis (Abstract).

With regard to claim 37, Lockhart discloses a method of analyzing a nucleic acid sample from a blood sample comprising:

a) amplifying mRNA from the nucleic acid sample to generate an amplified sample (Figure 1; p. 1679, col. 1 'RNA preparation for hybridization' heading);

b) labeling said amplified sample (Figure 1; p. 1679, col. 1 'RNA preparation for hybridization' heading);

c) hybridizing the amplified sample to an array of nucleic acid probes to generate a hybridization pattern (Figure 1; p. 1679, col. 2, 'array hybridization and scanning'); and

d) analyzing the hybridization pattern (Figure 1; p. 1679, col. 2 'quantitative analysis of hybridization patterns and intensities' heading).

With regard to claim 38, Lockhart discloses an embodiment of claim 37, wherein said amplifying step comprises hybridizing an extendable primer comprising oligo dT to said nucleic acid sample, extending said primer with a reverse transcriptase to make cDNA and amplifying said cDNA (p. 1679, col. 1, 'RNA preparation for hybridization' heading, where 1 microgram of poly (A)+ RNA was converted into double stranded cDNA using a cDNA synthesis kit with an oligo dT primer incorporating a T7 RNA polymerase promoter site).

With regard to claim 42, Lockhart discloses an embodiment of claim 37, wherein the hybridization pattern is analyzed to determine an expression profile for said nucleic acid sample

(Figure 1 and 2, p. 1679, col. 2 'Higher density arrays containing 65,000 probes for over 100 murine genes' heading, paragraph 2, where experiments were conducted to determine the proper number of redundantly spotted oligonucleotides necessary for efficient measurement of expression changes at low levels).

Regarding claim 38, Lockhart does not teach cDNA synthesis wherein globin mRNA is specifically blocked during amplification or that the sample is obtained from blood. Rampersad teaches a method for inactivating undesirable members in a sample which includes desirable and undesirable sequences in a mixture (Abstract).

With regard to claim 37, Rampersad discloses the blocking of amplification of a specific mRNA comprising hybridizing a reduction oligonucleotide to at least one mRNA in the sample (example 1, col. 5, lines 9-61, where RNA treated with the oligonucleotide blocker plus or minus oligonucleotide blocker and RNAse H were used as templates in reverse transcription reactions to generate cDNA, with the oligonucleotide blocker priming cDNA synthesis).

With regard to claim 39, Rampersad discloses the blocking of amplification of mRNA is blocked by hybridization of one or more blocking molecules to one or more transcripts prior to extending said extendable primer with reverse transcriptase (example 1, col. 5, lines 9-61, where RNA treated with the oligonucleotide blocker plus or minus oligonucleotide blocker and RNAse H were used as templates in reverse transcription reactions to generate cDNA, with the oligonucleotide blocker priming cDNA synthesis).

Regarding claim 37, Neither Lockhart or Rampersad teaches the blocking of globin mRNA specifically or obtaining a sample specifically from blood and neither teach the specific sequences of globin that are targeted for reduction from the sample(s). Kempe teaches the

extraction of RNA from blood obtained from rabbit reticulocytes, establishing that blood can be a good source of RNA, and particularly of the globin sequence (col. 27, lines 39-60). Baker teaches that overabundance of specific highly expressed sequences in the construction of libraries can be addressed through the inactivation and degradation of overabundant mRNA species (col. 5, lines 5-24).

With regard to claim 12, Baker discloses an embodiment of claim 10, wherein a plurality of different species of oligonucleotides are used and each species is complementary to a globin mRNA (col. 5, lines 5-24).

With regard to claim 37, Adams discloses an embodiment wherein the reduction oligo is selected from the group consisting of an oligonucleotide consisting of SEQ ID NO:1, an oligonucleotide consisting of SEQ ID NO:2 and an oligonucleotide consisting of SEQ ID NO:3 (see attached results, where sequences comprising alpha 1 globin (SEQ ID 1), alpha 2 globin (SEQ ID 2) and beta globin (SEQ ID 3) are disclosed by Adams and any one of these could be selected and be expected to function; see also attached sequence results, entry 4, where AA342142 discloses the 3' end of a cDNA sequence obtained from spleen which is similar to alpha 1 globin, with sequence identity of 100% across the 23 nucleotides comprising SEQ ID NO:1; also see attached sequence results, entry 4, where AA342142 discloses the 3' end of a cDNA sequence obtained from liver which is similar to alpha 2 globin, with sequence identity of 100% across the 23 nucleotides comprising SEQ ID NO:2; also see attached sequence results, entry 4, where AA342142 discloses the 3' end of a cDNA sequence obtained from spleen which is similar to beta globin, with sequence identity of 100% across the 26 nucleotides comprising SEQ ID NO:3).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have applied the teachings of Rampersad to the method of hybridization analysis taught by Lockhart to arrive at the claimed invention with a reasonable expectation for success. As taught by Rampersad, the term "undesired nucleic acids is used herein to denote nucleic acids in a sample that interfere with the use of the sample. Undesirable nucleic acids may include known or abundant members of a nucleic acid family that block the study, isolation or processing of desired novel or less abundant members of the same family" (col. 2, lines 30-34). In this case, the method is applied to a nucleic acid sample obtained from blood. As taught by Kempe and Baker, that blood is a source of globin mRNA and that "in many cells or tissues a particular mRNA species represents the vast majority of total mRNA. For example, abundant mRNAs such as those encoding globin, immunoglobulins and ovalbumin may constitute as much as 50-90% of the total poly(A)+ cytoplasmic RNA isolated from certain cell types" (col. 5, lines 7-14). Therefore, considering that one of the characteristics of an 'undesired nucleic acid' as disclosed by Rampersad included known or abundant members of a nucleic acid family and considering the level of abundance of globin mRNA, one of ordinary skill in the art would have considered targeting and depleting the globin mRNA sequence(s) from nucleic acid samples derived from blood.

Furthermore, when considering the step of analyzing the nucleic acid sample derived from blood, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have applied the enriched sample derived from blood to a microarray as disclosed by Lockhart. As noted by Lockhart, "array-based methods that involve the spotting of multiple clones or cDNAs onto nylon membranes or modified glass microscope slides have

been described. These approached have the inherent advantages of being parallel with direct and rapid readout of hybridization results" (p. 1675, col. 1). Furthermore, Lockhart teaches that arrays can also be generated through "oligonucleotides chemically synthesized in a highly combinatorial fashion directly on a solid substrate" and also notes that "because oligonucleotide probes for each gene are specifically chosen and synthesized in known locations on the arrays, the hybridization patterns and intensities can be interpreted in terms of gene identity and relative amount with no additional sequencing and characterization" (p. 1676, col. 2). The parallel nature of microarray hybridization provides the benefit of savings in time and in reagents. However, Lockhart also notes a few inconsistencies in hybridization data, noting "in a few instances, the PM probe is not significantly brighter than its MM partner because of cross-hybridization with other members of the complex RNA population" (p. 1677, col. 1). Lockhart also notes that "because the patterns are highly reproducible and because detection does not depend only on a single probe per RNA, infrequent cross-hybridization of this type does not preclude sensitive and accurate detection of even low-level RNAs" (p. 1677, col. 1). However, considering the abundance of globin mRNA within the blood and the potential for cross-hybridization or for non-specific background due to the abundance of the globin message within a blood sample, the selective removal of the abundant message through the technique disclosed by Rampersad would provide for an additional level of assurance that the globin mRNA message would not interfere with the sensitive detection of mRNA at all levels of expression within the blood sample.

In the recent court decision *In Re Deuel* 34 USPQ 2d 1210 (Fed. Cir. 1995), the Court of Appeals for the Federal Circuit determined that the existence of a general method of identifying

a specific DNA does not make the specific DNA obvious. Regarding structural or functional homologs, however, the Court stated,

"Normally, a *prima facie* case of obviousness is based upon structural similiarity, i.e., an established structural relationship between a prior art compound and the claimed compound. Structural relationships may provide the requisite motivation or suggestion to modify known compounds to obtain new compounds. For example, a prior art compound may suggest its homologs because homologs often have similar properties and therefore chemists of ordinary skill would ordinarily contemplate making them to try to obtain compounds with improved properties (see page 9, paragraph 4 of attached ref)."

Since the claimed primers simply represent structural homologs, which are derived from sequences suggested by the prior art as useful for primers and probes for the suppression of expression of globin genes, and concerning which a biochemist of ordinary skill would attempt to obtain alternate compounds with improved properties, the claimed primers and probes are *prima facie* obvious over the cited references in the absence of secondary considerations. Furthermore, considering the explicit teaching in the instant specification, noting, "the DNA bait can be designed to hybridize to part or all of the RNA to be hydrolyzed. To sever the 3'-polyA tail from the rest of an mRNA, an oligonucleotide directed to a region upstream or 5' of the polyA tail may be used." The instant specification also teaches "the reduction oligo hybridized to the region that is within 50, 100 or 200 bases of the 5' end of the poly(A) tail. Hybrids of greater length may be used to generate more extensive hydrolysis. Longer DNA bait could comprise, for example: multiple oligonucleotides hybridizing to different regions of the RNA to be hydrolyzed (p. 5 of PgPub, paragraph 50). Referring specifically to the reduction of globin specific mRNA, the instant specification teaches "the reduction oligos are designed that are complementary to a region in the 3' region of one or more globin genes" (p. 9 of PgPub, paragraph 69. While the instant specification exemplifies the use of the specific reduction oligonucleotides consisting of SEQ ID NO:1 (alpha 1), SEQ ID NO:2 (alpha 2) and SEQ ID NO:3 (beta), the specification does not provide specific teaching comparing these targeted locations as providing unexpected results over sequences targeted to a different region in each of

these mRNAs. Therefore, as the specification teaches that the specific location of the targeting sequence is not always restricted to only to a specific targeted location and there are no specific teachings of unexpected results associated with the specific sequences consisting of SEQ ID NO:1, 2 and 3, there is a reasonable expectation that any sequence targeting a specific sequence would be successful in depleting the targeted RNA messages, in the absence of unexpected results or other secondary considerations.

8. Claim 40-41 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lockhart et al. (1996, Nature Biotech, vol. 14, p. 1675-1680) in view of in view of Rampersad et al. (US Patent 5,830,712; November 1998), Kempe (US Patent 4,661,450; April 1987), Baker et al (US Patent 5,643,780; July 1997) and Adams et al. (Nature, 1995, supplement, vol. 377, p. 3-17) as applies to claims 37-39 and 42 above and further in view of Mugasimangalam et al. (US Patent 6,544,741; September 2003). Lockhart teaches hybridization to small, high-density oligonucleotide arrays, designed using a combination of photolithography and oligonucleotide synthesis (Abstract).

Lockhart teaches the limitations of claims 37-38 and 42 as recited in the 102 rejection stated above. However, Lockhart does not teach the use of blocking molecules. Mugasimangalam discloses methods and materials for normalizing nucleic acids, using a technique called 'prime and kill' (col. 3, lines 44-51).

With regard to claim 40, Mugasimangalam discloses an embodiment of claim 38, wherein said one or more blocking molecules are peptide nucleic acids (col. 7, lines 48-51, where the killer primer can comprise peptide nucleic acids containing DNA analogs with amide backbone linkages).

With regard to claim 41, neither Mugasimangalam nor Lockhart teaches the application of the technique to specific globin target molecules. Kemp et al.teaches an embodiment of claim 39, wherein said one or more blocking molecules hybridize to a globin mRNA selected from the group consisting of alpha-1 globin, alpha-2 globin and beta globin (col. 27, lines 39-60, where beta globin is extracted from blood).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to incorporate the "killer primer" method taught by Mugasimangalam into the method of inactivation and blocking taught by Rampersad with a reasonable expectation for success. Mugasimangalam teaches that "the methods and materials provided herein efficiently reduce the redundancy of highly expressed genes, and increase the relative amount of transcripts of rarely expressed genes" (col. 7, lines 5-13). On the other hand, as taught by Rampersad, the term "undesired nucleic acids is used herein to denote nucleic acids in a sample that interfere with the use of the sample. Undesirable nucleic acids may include known or abundant members of a nucleic acid family that block the study, isolation or processing of desired novel or less abundant members of the same family" (col. 2, lines 30-34). As both Rampersad and Mugasimangalam teach related methods for the reduction of abundant target sequences in complex mixtures of oligonucleotides, it would have been obvious to one of ordinary skill in the art at the time the invention was made to incorporate the teachings of Mugasimangalam, including peptide nucleic acid (PNA) molecules as the blocking molecules for the depletion of targeted sequences. One of ordinary skill would have recognized the benefit of modifying a technique to fit a given set of experimental conditions and would have therefore been motivated

to consult similar methods in the art in order to arrive at the most optimal experimental conditions to achieve the stated goal with a reasonable expectation for success.

12. Claim 43 is rejected under 35 U.S.C. 103(a) as being unpatentable over Lockhart et al. (1996, Nature Biotech, vol. 14, p. 1675-1680) in view of in view of Rampersad et al. (US Patent 5,830,712; November 1998), Kempe (US Patent 4,661,450; April 1987), Baker et al (US Patent 5,643,780; July 1997) and Adams et al. (Nature, 1995, supplement, vol. 377, p. 3-17) as applies to claims 37-39 and 42 above and further in view of Augello et al. (US Patent 6,602,718; August 2003). Lockhart teaches hybridization to small, high-density oligonucleotide arrays, designed using a combination of photolithography and oligonucleotide synthesis (Abstract).

With regard to claim 43, Augello discloses an embodiment of claim 37, wherein said nucleic acid sample is isolated from a blood sample that was collected in a container containing an RNA stabilizing agent selected from the group consisting of cationic compounds, detergents, chaotropic salts, ribonuclease inhibitors, chelating agents, phenol, chloroform, acetone, alcohols, mercapto-alcohols, dithiothreitol (DTT) and mixtures thereof (col. 6, line 51 to col. 7 line 18).

Furthermore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to incorporate the teachings of Augello into the method taught by Rampersad with a reasonable expectation for success. As taught by Augello, upon evaluating the stabilization of nucleic acids obtained using the collection container with RNA stabilizers, "The real-time PCR results show that in the unpreserved EDTA blood, the transcript level decreases over time (indicated by the increasing Ct value in the TaqMan analysis) up to a degree of degradation after 7 to 10 days at which point the mRNA is no longer detectable. On the other

hand the GAPDH mRNA in the preserved samples does not show any decrease in copy number" (col. 11, lines 26-33). Therefore, the inclusion of the RNA stabilizer and collection container taught by Augello provides the benefit of increased stability over time, allowing for analysis of RNA samples over a longer time frame. One of ordinary skill in the art would have recognized the benefit of blood collection in the presence of RNA stabilizers, who would have therefore been motivated to incorporate the teachings of Augello to the method taught by Rampersad with a reasonable expectation for success.

Response to Arguments

2. Applicant's arguments with respect to claims 1-7, 11-26, 28, 30-34, 37, 40-43 and 45 have been considered but are moot in view of the new ground(s) of rejection.

Relevant prior art

Kothapalli et al. (BMC Bioinformatics, 2002, vol. 3, biomedcentral.com/1471-2105/3/22) discloses a detailed analysis of data obtained from two different microarrays, including sequence fidelity, confirmation of differential expression and specificity of cDNA probes.

Conclusion

Claims 1-7, 11-26, 28, 30-34, 37-43 are rejected.

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Stephanie K. Mummert, Ph.D., Ph.D. whose telephone number is 571-272-8503. The examiner can normally be reached on M-F, 9:00-5:30.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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SKM

JEFFREY FREDMAN
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5/17/07